REMARKS

The present invention relates to regulatory T cells (Treg cells) and methods of long-term, culture-expanding, activating and using same in immunotherapy and for the suppression of autoimmune responses.

Claims 1-25 are presently pending in the present application. Claims 12-25 have been withdrawn from consideration as being drawn to a non-elected invention. Therefore, claims 1-11 are currently under examination.

By way of the present Amendment, claims 1, 4, 5, and 10 have been amended as more fully discussed below. Claim 4 has been amended to recite a double column purification procedure. Claim 5 has been amended to recite $CD4^+CD25^+$ cells. Claim 10 has been amended to correct a typographical error. Now new matter has been added by way of these amendments.

Claims 26-29 have been added. Support for claim 26 relating to a second generation lineage depletion protocol is found in paragraph 91, page 27. Support for claims 27 and 28 relating to the ratio of antibodies to CD3 and CD28 is found in paragraph 86, on page 25-26. Support for claim 29 relating to long term down-regulatory suppressor function is found in paragraph 73, on page 21. Thus, no new matter has been added by way of these new claims.

Objection to claims 1

The Examiner has objected to claim 1 for informal matters. Claim 1 has been amended to delete the second recitation of the term "producing".

Response to Rejections Under 35 U.S.C. 112, Second Paragraph

Claims 5-10 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. The Examiner contends that it is unclear as to what the two steps encompass with respect to performing a second-generation lineage depletion protocol using two steps.

The present invention is partly based on the discovery of a selective culture methodology which generates a potent suppressor cell line. The methodology encompasses an *ex vivo*, long-term, culture-expanding human $CD4^+CD25^+$ for producing therapeutic human Treg cells with enhanced suppressive activity. The culture-expanding method includes the activation of isolated $CD4^+CD25^+$ cells with a cleavable cell-sized, antibody-coated, magnetic microbeads.

Claim 5 has been amended to indicate that the $CD4^+CD25^+$ cells are activated with a cleavable cell-sized, antibody-coated, magnetic microbead. Support for this amendment is found throughout the specification (*See, e.g.*, paragraph 26 beginning on page 8 and paragraph 188 beginning on page 52). Applicants submit that amended claim 5 more clearly defines the invention whereby the isolated $CD4^+CD25^+$ cells are culture expanded by incubating the cells with microbeads coated with antibodies such as anti-CD3 and anti-CD28.

In view of the amendment to claim 5, Applicants have added claim 26 to the application. Specifically, claim 26 is directed to a method comprising a second generation lineage depletion protocol using two steps, wherein the first step encompasses isolating a population of $CD25^+$ T cells from a sample using anti-CD25. The second step includes depleting $CD8^+$ T cells from the population of isolated $CD25^+$ T cells using anti-CD8 microbeads. This two step process is useful for the generation of improved suppressor T cells as compared to cells generated from using prior art methods. Support for this amendment is found in paragraph 91, on page 27, and in original claim 5. Applicants assert that no new matter has been added by way of these amendments.

Accordingly, Applicants respectfully request that the rejection of claims 5-10 under 35 U.S.C. § 112, second paragraph, be reconsidered and withdrawn.

Rejection of claims 1-4 and 11 pursuant to 35 U.S.C. §102(e)

The Examiner has rejected claims 1-4 and 11 under 35 U.S.C. § 102(e) as being anticipated by Schuler et al. (U.S. Patent Application Pub. No. 2005/0101012). The Examiner is of the opinion that Schuler teaches isolating $CD4^+$ T cells from human PBMC and isolating the $CD4^+CD25^+$ T cells from the pure untouched $CD4^+$ T cells using CD25 microbeads. The Examiner asserts that Schuler anticipates these claims. Applicants have amended the claims and therefore believe this rejection no longer applies.

It is hornbook law that "[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." MPEP §2131 (quoting *Verdegaal Bros. v. Union Oil Co. of Calif.*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)). "The identical invention must be shown in as complete detail as is contained in the . . . claim." *Id.* (quoting *Richardson v. Suzuki Motor Co.*, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989) (emphasis added). Therefore, Schuler must describe each and every

element of the claims in order to anticipate these claims under 35 U.S.C. §102(e), and this reference does not satisfy this requirement.

Claim 1 has been amended to indicate that the method of producing therapeutic human Tregs with enhanced suppressive activity includes isolating a population of human $CD4^+CD25^+$ cells from a sample of $CD4^+$ T cells using a lower titer of anti-CD25 antibody in a modified MACS purification procedure comprising a double column purification procedure. Applicants contend that this amendment to claim 1 is supported by the as-filed application. For example, paragraph 85, on page 25, and paragraph 185, on page 52 of the specification discloses the double column procedure which is described as being a more stringent purification strategy that lead to the isolation and generation of more potent suppressor cell lines. Furthermore, paragraph 88, on page 26 indicates that lower titers of anti-CD25 magnetic microbeads and re-purification over a second column greatly facilitated the generation of Tregs with potent suppressive capabilities.

The Examiner states that the teachings of Schuler inherently includes washing and re-eluting the cells over a second column as evidenced by the protocol for the CD25 Microbeads from Miltenyi Biotech. The Examiner is improperly relying on two references in making this rejection (*i.e.* Shuler and the Miltenyi Biotec CD25 MicroBeads protocol ("the Miltenyi protocol")). Applicants understand that in some instances, a rejection under 35 U.S.C. §102 based on multiple references has been held to be proper when the extra references are cited to:

(A) Prove the primary reference contains an "enabled disclosure;"
(B) Explain the meaning of a term used in the primary reference; or
(C) Show that a characteristic not disclosed in the reference is inherent.

The Examiner contends that re-eluting the cells over a second column is inherent in view of the Miltenyi protocol. However, Schuler does not refer to the Miltenyi protocol. Instead, Schuler cites to Miltenyi Biotec for the purpose of providing a source for their kit. Nowhere does Schuler state that the Miltenyi protocol was either followed or modified. Thus, the Examiner's inclusion of the Miltenyi protocol in her rejection of the claims under 35 U.S.C. §102(e) is improper under the law and must be withdrawn.

The Examiner contends that Schuler does not need to specifically recite a double column purification step because it is inherent in the Miltenyi protocol. The Miltenyi protocol is an improper citation and therefore cannot be relied on to support this rejection. Further, nowhere

in Schuler alone, inherently or not, is there a disclosure for using a low titer of anti-CD25 in a modified MACS purification procedure. MPEP § 2112 provides:

> "In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristics necessarily flows from the teachings of the prior art. (emphasis in original)

The Examiner has failed to meet the burden established by the MPEP. Schuler does not teach the use of a low titer of anti-CD25. Schuler does not inherently teach the presently claimed invention. Schuler merely teaches the isolation of $CD25^+$ cell using CD25 microbeads. In fact, Schuler does not even disclose the procedure used to isolate the cells. Rather, Schuler simply makes a reference to the isolation kit of Milteyi Biotech when describing the source of the kit. Nowhere does Schuler indicate any particular procedure or even whether the procedure was carried out according to manufacture's protocol or modified in someway. Therefore, there exists no fact and/or technical reasoning in Schuler to support the Examiner's contention that re-eluting the cells over a second column is inherent, and the Miltenyi protocol cannot be used to support this rejection.

Schuler does not teach each and every element of claim 1 as amended herein, and therefore does not anticipate this claim and dependent claims therefrom. Schuler does not teach a method of using a lower titer of anti-CD25 in a modified MACS purification process comprising a double column purification procedure. The present invention is partly based on the discovery that by performing more stringent purification methodologies, the more potent and reproducible suppressor T cells were isolated. As a result, experiments directed to anti-CD25 microbead titration were conducted, which led to the development of the double column purification protocol. As described in Example 8, using a lower titer of anti-CD25 coated microbeads led to isolation of T cells exhibiting more suppressor activity compared to the activity corresponding to T cells isolated using prior art methods.

Without the initial anti-CD25 microbead titration experiments, the development of the double column purification procedure would not have been evident. Without the use of the claimed method, a population of T cells with potent suppressor activity or otherwise enhanced suppressive activity would not have been successfully isolated.

Further, Schuler in view of the Miltenyi protocol fails to teach each and every element of the claimed invention. Specifically, Schuler fails to teach low titers of anti-CD 25, and the Miltenyi protocol, even if it was a proper reference, does not cure this deficiency. Applicants contend that using a low titer of anti-CD25 in a modified MACS purification procedure comprising a double column procedure of the present invention is an improvement over prior art methods because such a procedure is a more stringent purification methodology. As such, the double column purification procedure is useful in view of the fact that it is advantageous to isolate the $CD25^{bright}$ subset of $CD4^+CD25^+$ cells in order to detect suppressor activity. This is because it was observed that contaminating of $CD25^{dim}$ cells in $CD25^+$ fractions grew faster and can overgrow the $CD25^{bright}$ cells, and thereby preclude the full manifestation of suppressor cell function (*See, e.g.*, Example 8). It was observed that $CD25^{dim}$ cells exhibited a lower suppressive activity than $CD25^{bright}$ cells (*See, e.g.*, paragraph 24, page 8).

In some instances, the result of using the double column purification methodology of the invention is the generation of $CD4^+CD25^+$ cells that exhibited potent functional suppressor activity (*e.g.*, >90% inhibition). When assayed, the culture-expanded human suppressor cells of the present invention are capable of about >90% suppression of an MLR, either with fresh $CD4^+$ cells or cultured $CD4^+CD25^-$ cells as responding T cells (*See, e.g.*, Example 8).

Applicants respectfully submit that the claims, as amended herein, are not anticipated by Schuler for the reasons set forth above, and request reconsideration and withdrawal of the rejection pursuant to 35 U.S.C. §102(e).

Rejection of claims 1-3, 5 and 11 pursuant to 35 U.S.C. §102(e)

The Examiner has rejected claims 1-3, 5 and 11 under 35 U.S.C. § 102(e) as being anticipated by Roncarolo et al. (U.S. Patent Application Pub. No. 2004/0173778). The Examiner is of the opinion that Roncarolo teaches the isolation of suppressive $CD4^+CD25^+$ T cells comprising purifying $CD4^+$ T cells from PBMC using anti-CD4-coupled microbeads, separating $CD25^+$ cells from the isolated $CD4^+$ T cells using PE coupled anti-CD25 monoclonal antibodies followed by addition of anti-PE coupled magnetic beads. Therefore the Examiner asserts that Roncarolo anticipates these claims. Applicants disagree with the Examiner for the following reasons.

As an initial matter, Applicants submit that the deficiencies of Schuler discussed above, although not repeated here, are equally applicable to the instant rejection of claims 1-3, 5 and 11 under 35 U.S.C. §102(e) over Roncarolo. This is because nowhere does Roncarolo teach using a lower titer of anti-CD25 in a modified MACS purification procedure comprising a double column purification procedure. Similar to Schuler, Roncarolo isolates $CD4^+CD25^+$ T cells by way of a single purification procedure.

Moreover, nowhere does Roncarolo discuss a cell line that exhibits the potent functional suppressor activity (*e.g.*, >90% inhibition) exhibited by the cells generated from the claimed method. At best, it appears that Roncarolo discloses $CD4^+CD25^+$ T cells that are able to inhibit the proliferation of naïve $CD4^+CD25^-$ T cells by an average of 75%. Not only does Roncarolo not teach the same method of the presently claimed method, Roncarolo also does not arrive at the same cell population generated from using the claimed double column purification procedure.

Applicants respectfully submit that the claims, as amended herein, are not anticipated by Roncarolo for the reasons set forth above, and request reconsideration and withdrawal of the rejection pursuant to 35 U.S.C. §102(e).

Rejection of claims 6-10 pursuant to 35 U.S.C. §103(a)

The Examiner has rejected claims 6-10 under 35 U.S.C. § 103(a) as being unpatentable over Roncarolo et al. in view of Diehn et al., (2002, PNAS 99:11796-11801). Specifically, the Examiner contends that Diehn teaches the use of a 1:1 mixture of anti-CD3 and anti-CD28 coated activated polysterene beads, and therefore it would have been obvious to one of skill in the art to use the teachings of Roncarolo and Diehn to activate the suppressor regulatory T cells. Applicants respectfully traverse this rejection for the following reasons.

In making a case for obviousness, the Examiner must 1) determine the scope and contents of the prior art; 2) ascertain the differences between the prior art and the claims at issue; 3) resolve the level of ordinary skill in the pertinent art; and 4) evaluate evidence of secondary considerations (*See*, MPEP 2141).

When applying 35 U.S.C. § 103, the following tenets of patent law must be followed: 1) the claimed invention must be considered as a whole; 2) the references must be considered as a whole and must suggest the desirability and thus the obviousness of making the

combination; 3) the references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and 4) reasonable expectation of success is the standard with which obviousness is determined (*See*, MPEP 2141).

As an initial matter, claims 6-10 ultimately depend from claim 1. Therefore, the amendment to claim 1 with respect to a low titer of anti-CD25 in a modified MACS purification procedure comprising a double column purification procedure is an element that must be considered in rejecting claims 6-10. The deficiencies of Roncarolo discussed above, although not repeated here, are equally applicable to the instant rejection. As such, the amendment to claim 1 which encompasses the use of a low titer of anti-CD25 in a modified MACS purification procedure comprising a double column purification procedure renders Roncarolo inapplicable. Therefore, Diehn is required to teach this purification procedure in order to cure the deficiencies of Roncarolo.

Applicants submit that nowhere does Diehn discuss specifically $CD4^+CD25^+$ T cells, let alone methods for producing therapeutic human T regulatory cells with enhanced suppressive activity. Rather, consistent with the Examiner's reading of Diehn, Diehn is merely a generic teaching of the requirement of both the antigen-specific T cell receptor and a second coreceptor CD28 for optimal activation of T cells. Therefore, Roncarolo in combination with Diehn cannot render the pending claims *prima facie* obvious.

Applicants respectfully submit that this rejection under 35 U.S.C. § 103 is overcome by reliance on the limitation of the double column purification procedure. Nowhere does Diehn disclose a double column purification procedure for isolating T cells. As such, there is no suggestion or motivation for isolating Tregs using a lower titer of anti-CD25 in a modified MACS purification procedure comprising a double column purification procedure as claimed in claim 1. Accordingly, there is no reasonable expectation that a population of therapeutic human Treg cells with enhanced suppressive activity would be generated using the methods disclosed by Diehn or for that matter Roncarolo in combination with Diehn.

In addition, the combined teachings of Ronacarolo and Diehn would teach away from the presently claimed method. Applicants have demonstrated a method for generating a population of Treg cells that exhibit enhanced suppressive activity. For example, the specification discloses that the double column purification methodology makes it possible to generate T cells that exhibit potent functional suppressor activity (*e.g.*, about >90% suppression

of an MLR, either with fresh $CD4^+$ cells or cultured $CD4^+CD25^-$ cells as responding T cells, (*See, e.g.*, Example 8). Applicants submit that none of the cells disclosed by Ronacarolo and Diehn, exhibit this suppressor activity profile.

It is also submitted that the enhanced suppressor activity of the cells generated using the double column purification procedure was an unexpected result. This is because if one considers the art as a whole, including the teachings of Ronacarolo and Diehn, one of skill in the art would be lead to believe that a population of T cells exhibiting potent suppressor activity could not be made using a double column purification. Ronacarolo goes to the level of cloning a population of T cells using limited dilution procedures to generate a relatively pure population. Ronacarolo does not indicate that these clonal population of T cells exhibited the high level of suppressor activity compared to the level observed by Applicants using a double column purification procedure.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the Examiner's rejection pursuant to 35 U.S.C. §103(a). It is believed that the amendment to claim 1 renders the rejection of claims 6-10 under 35 U.S.C. §103(a) moot.

Rejection of claim 1 for Nonstatutory Obviousness-type Double Patenting

The Examiner has provisionally rejected claim 1 under 35 U.S.C. §101 on the grounds of nonstatutory obviousness-type double patenting. The Examiner is of the opinion that claim 1 is unpatentable over claims 1-3, 10 and 14 of copending Application No. 11/226,168.

Applicants request that the provisional nonstatutory double patenting rejection be placed in abeyance until claims have actually issued or are deemed allowable in one of the applications.

Summary

Applicant respectfully submits that each rejection of the Examiner to the claims of the present application has been overcome or is now inapplicable, and that the claims are now in condition for allowance. Reconsideration and allowance of these claims is respectfully requested at the earliest possible date.

Respectfully submitted,

BRUCE BLAZAR ET AL.,

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(Date)

By: _____

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